CLAIMS:

- A method of producing a refolded, inactive HCV NS2/3 protease, comprising the steps of:
 - a) isolating said protease in the presence of a chaotropic agent;
 - b) refolding said isolated protease by contacting it with a reducing agent, and lauryldiethylamine oxide (LDAO) in the presence of reduced concentration of said chaotropic agent or a polar additive.
- 2. The method according to claim 1, wherein said LDAO is at a final concentration at, or above critical micelle concentration.
- 3. The method according to claim 2, wherein said LDAO is at a final concentration between 0.003% and 1%.
- 4. The method according to claim 3, wherein said LDAO is at a final concentration between 0.03% and 1%.
- 5. The method according to claim 4, wherein said LDAO is at a final concentration of 1%.
- 6. The method according to claim 1, wherein in step a) said chaotropic agent is selected from the group consisting of: guanidine-HCl, guanidine or urea.
- 7. The method according to claim 6, wherein said chaotropic agent is at high concentration between 5M and 8M.
- 8. The method according to claim 7, wherein said chaotropic agent is guanidine or guanidine-HCl, each at a final concentration of 6M or urea at a final concentration of 8M.
- 9. The method according to claim 8, wherein said chaotropic agent is 6M guanidine-HCI.
- 10. The method according to claim 1, wherein in step b), the chaotropic agent or polar additive is selected from the group consisting of: guanidine, guanidine-HCI, urea and arginine-HCI.
- 11. The method according to claim 10, wherein guanidine-HCl or arginine-HCl is used.
- **12.** The method according to claim 11, wherein arginine-HCl is used.
- 13. The method according to claim 12, wherein said arginine-HCl is at a final concentration between 0.25M and 2M.
- 14. The method according to claim 13, wherein said arginine-HCl is at a final

- concentration between 0.5M and 1M.
- 15. The method according to claim 14, wherein said arginine-HO is at a final concentration of 0.5M.
- **16.** The method according to claim 1, wherein the reducing agent is selected from the group consisting of TCEP and DTT.
- 17. The method according to claim 16, wherein the reducing agent is TCEP at a final concentration of 5mM.
- **18.** The method according to claim 1, wherein said protease is isolated from cellular inclusion bodies.
- **19.** The method according to claim 1, wherein said refolding is carried out by dialysis or by gel filtration to yield a purified NS2/3 protease.
- 20. The method according to claim 19, wherein said refolding is carried out by gel filtration.
- 21. The method according to claim 1, wherein said NS2/3 protease is the full length NS2/3 protease or a truncation thereof having as its N-terminal residue any one amino acid from amino acid 810 to amino acid 906.
- 22. The method according to claim 21, wherein said NS2/3 protease has the minimal amino acid sequence from residues 904 to 1206 of the HCV 1b-40 full-length NS2/3 protease.
- 23. The method according to claim 22, wherein said NS2/3 protease is consisting of a truncated NS2/3 protease as defined according to SEQ ID. NO: 10.
- 24. A method for producing an active NS2/3 protease further comprising:
 - c) diluting said refolded inactive NS2/3 protease as defined in claim 1, in a medium containing an activation detergent to induce auto-cleavage of said NS2/3 protease.
- 25. The method according to claim 24, wherein said LDAO is diluted at a final concentration equal or below 0.1%
- 26. The method according to claim 24, wherein in step c) glycerol is further added.
- 27. The method according to claim 26, wherein said glycerol is at a final concentration of between 10% and 50%.
- 28. The method according to claim 24, wherein the activation detergent is selected from the group consisting of: CHAPS, Triton X-100, NP-40 and n-dodecyl-β-D-maltoside.
- 29. The method according to claim 28, wherein the activation detergent is at a final concentration between 0.1% and 1%.

- 30. The method according to claim 29, wherein the activation detergent is CHAPS.
- 31. The method according to claim 29 wherein the activation detergent is n-dodecyl-β-D-maltoside.
- **32.** A method for measuring the auto-cleavage activity of a/NS2/3 protease further comprising:
 - d) incubating the active NS2/3 protease produced by the method of claim 24 for sufficient time to induce auto-cleavage of the NS2/3 protease and produce cleavage products or fragments thereof; and
 - e) measuring the presence or absence of uncleaved NS2/3 protease, cleavage products or fragments thereof.
- 33. The method according to claim 32, wherein step d) is carried out at a temperature between 15°C and 30°C.
- 34. The method according to claim 33, wherein step d) is carried out at a temperature between 15°C and 25°C.
- **35.** The method according to claim 34, wherein step d) is carried out at room temperature.
- **36.** An assay for screening a potential inhibitor of the auto-cleavage activity of an active NS2/3 protease comprising:
 - a) carrying out the method according to claim 32 in the presence of, or absence of the potential inhibitor;
 - b) comparing the amount of uncleaved NS2/3 protease, cleavage products or fragments thereof, in the presence of, or absence of the potential inhibitor.
- An isolated polypeptide consisting of a full length NS2/3 protease or a truncation thereof having as its N-terminal residue any one amino acid from amino acid 810 to amino acid 906.
- 38. An isolated polypeptide consisting of a truncation of an HCV NS2/3 protease, wherein said truncated NS2/3 protease has the minimal amino acid sequence from residues 904 to 1206 of the HCV 1b-40 full-length NS2/3 protease.
- 39. An isolated polypeptide consisting of a truncated NS2/3 protease as defined according to SEQ ID. NO: 4 or 10.
- **40.** An isolated polypeptide consisting of an amino acid sequence that has 90% identity over its length compared to the polypeptide according to claim 39.
- 41. An isolated polypeptide consisting of a truncated NS2/3 protease selected from the



- group consisting of: a sequence as defined according to SEQ ID. NOs: 11, 12, 13, 14 and 15.
- 42. A composition comprising an isolated HCV N\$2/3 protease selected from full length NS2/3 protease, a truncation thereof or a sequence as defined according to SEQ ID NOs: 2, 4, 10, 11, 12, 13, 14 and 15, wherein said protease is in a solution comprising a sufficient concentration of LDAO to prevent auto-cleavage of said protease.
- 43. A peptide having the sequence SFEGQGWRLL (SEQ ID NO:21).
- 44. A peptide according to claim 43 wherein said peptide is a NS2/3 protease inhibitor.